

(μm) and width (μm) of the dividing cells were measured in different microscopic fields with ocular micrometer. Data were analysed statistically including mean value and standard error following [9].

3. Results

3.1 Mitotic Index and Abnormality Index

Mitotic index reflects the cell division frequency of an organ and is used to determine the root growth ratio as significant parameter. The decrease in mitotic index with increase in abnormality index and number of abnormal cells took place in root tips raised from seeds kept in increasing stressed conditions as compared to control set (Table 1). Treatment with PEG-6000 solution of -1.0 MPa water potential caused maximum number of abnormal cells with minimum mitotic index. The stage-wise abnormal cells of the root tips of different treated groups included chromosomal breakage at

prophase, C- metaphase, sticky chromosomes, vagrant chromosomes, disorientation of chromosomes and chromosomal breakage in metaphase, disturbed polarity and chromosomal breakage in anaphase (Table 1 and Figure 1). Although C-metaphase and sticky chromosomes were observed in the root tip meristems of all the treatments, the frequency of these abnormal cells was found to increase with increasing osmotic potential (Table 2).

3.2 Cell Length and Width

Table 1 shows that length of cells decreased gradually with the decreasing water potential. Though the increment in cell width was recorded at -0.1 MPa water potential, the width reduced gradually with lowering of water potential. Maximum reduction in cell size was noted in -1.0 MPa water potential.

Table 1: Changes in mitotic index, abnormality index and cell size (length and breadth) of root tip meristem of *Trigonella foenum-graecum* L. in response to PEG-6000 induced water deficit stress. Each value represents mean \pm S.E. (n=3).

Water potential of PEG-6000 solution (MPa)	Mitotic index (%)	Abnormality index (%)	Cell length (μm)	Cell breadth (μm)
0	40.938** \pm 0.79	0.583** \pm 0.23	31.8** \pm 0.13	22.36** \pm 0.09
-0.1	30.623** \pm 0.89	2.73** \pm 0.33	30.16** \pm 0.05	23.72** \pm 0.43
-0.5	28.152** \pm 0.70	5.521** \pm 0.59	26.96** \pm 1.2	21.11** \pm 0.58
-1.0	25.271** \pm 0.82	5.714** \pm 0.08	24.21** \pm 0.27	20.55** \pm 0.12

Table 2: Number of different types of chromosomal abnormalities at various stages of mitosis in root tip cells of *Trigonella foenum-graecum* L. with different water potentials of PEG-6000

Water potential (MPa)	Number of cells	Number of different types of chromosomal abnormalities										
		Prophase		Metaphase					Anaphase			
		Breakage	C-Metaphase	Sticky chromosome	Vagrant chromosome	Disorientation	Breakage	Sticky Chromosome	Breakage	Disturbed polarity	Chromosomal Bridge	Laggard Chromosome
0	1258	-	2	-	1	-	-	-	-	-	-	-
-0.1	1316	-	3	5	-	2	-	-	-	-	-	-
-0.5	1158	1	4	7	3	1	-	1	-	1	-	-
-1.0	1419	2	5	9	1	1	1	1	1	-	2	1

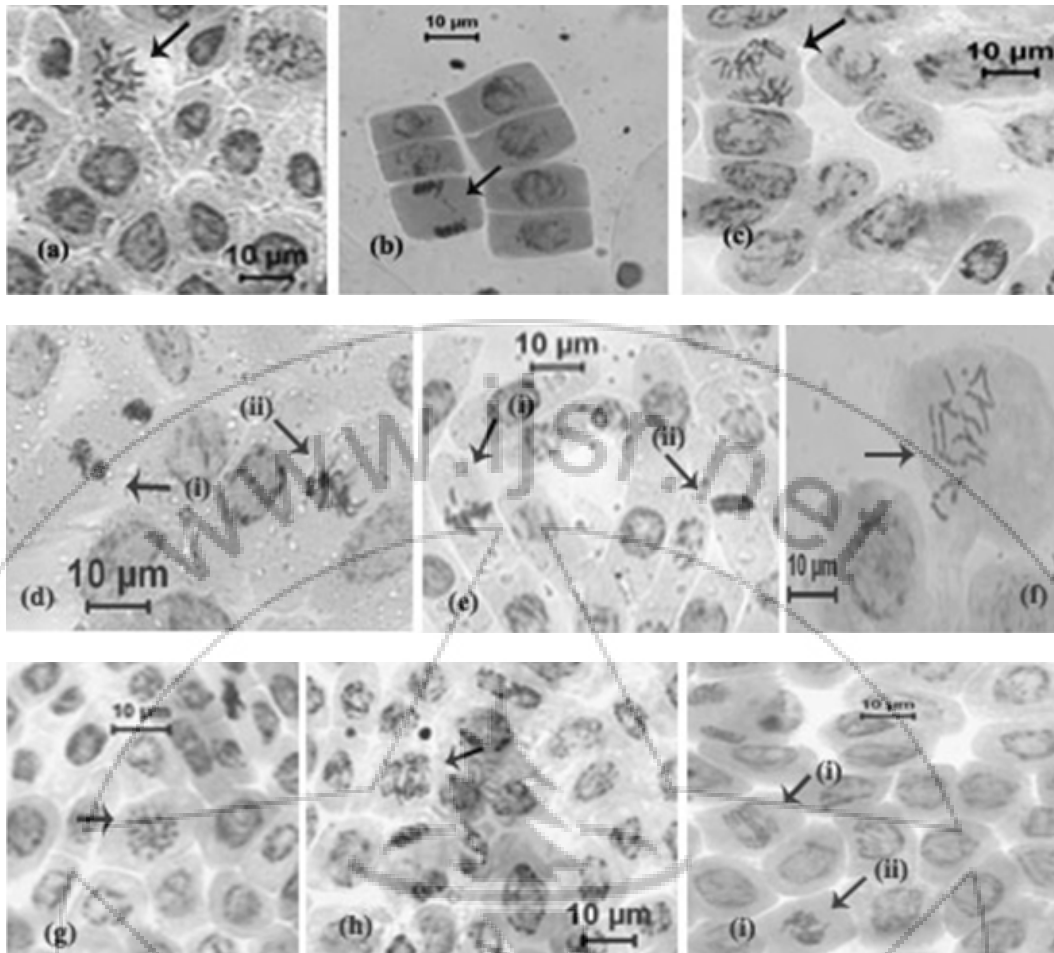


Figure 1: Photograph showing effects of water deficit stress induced by PEG – 6000 solutions of various water potentials on root tip meristem of *Trigonella foenum-graecum* L.

(a) C-metaphase (-0.1 MPa); (b) Laggard at anaphase (-1.0 MPa); (c) Disturbed polarity at anaphase (-0.5 MPa); (d)-(i) Sticky chromosome at anaphase (-0.5 MPa); (d)-(ii) Sticky chromosomes at metaphase (-0.5 MPa); e-(i) Vagrant chromosome at metaphase (-0.5 MPa); e-(ii) Sticky chromosomes at metaphase (-0.5 MPa); (f) C-metaphase with chromosomal breakage (-1.0 MPa); (g) Breakage at prophase (-1.0 MPa); (h) Chromosomal breakage at anaphase (-1.0 MPa); (i)-(i) Multiple bridge at anaphase (-1.0 MPa); (i)-(ii) Sticky bridge at late anaphase (-1.0 MPa);

4. Discussion

The water stress caused a mitodepressive effect on root tip cells of *Trigonella foenum-graecum* L. (Table 1). There are reports of rapid decrease in mitotic activity in roots after imposition of water stress in *Vicia faba* [3] and pea [10]. A similar response was found in soybean hypocotyl [11]. Similar types of cytological anomalies during meiosis were reported by Singh [12] in barley under water stress condition. In the life cycle of a plant a highly dehydration sensitive developmental phase comes immediately after germination [13], where many chromatin regulators act to trigger water stress-dependent growth arrest, which resembles the growth arrest during late embryogenesis in seed development [14]. Schuppler et al. [15] pointed out that water stress had induced a signal that increased phosphorylation of tyrosine at the active site of Cdc-2 kinase resulting accumulation of the inactive form of the enzyme

and consequently the progression into mitosis was inhibited. Some workers suggest that osmotic stress and regulation of cell cycle share common signaling pathways which can be influenced by ABA [16]. The present study showed different types of mitotic abnormalities in drought stressed root tip meristem of *Trigonella*. The pronounced effect spindle failure which caused C-metaphase, disorientation of chromosomes, disturbed polarity, laggard and vigrant chromosomes during cell division in root meristem.

It seems that the lower activity of Cdc-2 kinase due to drought stress may have caused impairment of spindle activity, because some workers suggest that at mitosis higher activity of this enzyme causes the structural changes of nuclear envelope disassembly, chromosome condensation and mitotic spindle assembly in yeast and animals [17-18]. Stickiness is a sign of toxic influence on the chromosomes probably leading to cell death [19]. Partial dissociation of nucleoproteins and alteration in the pattern of organization of chromosomes [20] or due to disturbances in cytochemically balanced reactions cause stickiness of chromosomes [21], which might have occurred in *T. foenum-graecum* L. due to water stress in cells. It may be possible that water deficiency may have caused some kind of gene mutation leading to incorrect coding of some non-histone proteins involved in chromosome organization. These proteins lead to stickiness. The chromosomal breakage indicates clastogenic effects. A secondary stress like oxidative stress in the condition of hyperosmolarity

develops due to higher amount of reactive oxygen species (ROS). ROS can have damaging effect on cellular structures and macromolecules especially DNA inducing numerous lesions that cause deletions, mutations and other lethal genetic effects [22].

The observed reduction in length and width of the cells (Table 1) was due to dehydration mediated cell shrinkage resulting consequent reduction in cellular volume [23].

5. Conclusion

The study provides the information that water deficit stress, given at the early stage, has an adverse impact on the fenugreek plant at adult stage, which may affect its genetic purity as well as yield potentiality by anomalous cytological behaviour.

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